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Abstracts

1 **Tuberculosis and Leprosy,** by ESMOND R. LONG, *Journal-Lancet*, Minneapolis, Nov. 1964, **84,** 11, 395-400.

Retrospective advances in the knowledge and control of tuberculosis and leprosy are described based on a symposium in 1937. Knowledge has since accumulated and includes that on a number of significant mycobacterial diseases, such as those due to *Mycobacterium kansasii*, *M. balnei*, *M. ulcerans*, and *M. fortuitum*, and the *Battey bacillus*. (Recently the powerful chemotherapeutic efficacy of 'B663', a new rimino-compound discovered by V. C. Barry, has been reported from Uganda as definitive against *M. ulcerans*. Strangely, B663 is concurrently being tried against leprosy, and there are previous reports of its action against *M. tuberculosis*. Editor). Dr. Long rightly looks to study of the other mycobacterial infections to provide leads for the difficult problem of isolation and cultivation of the infecting agent of leprosy, and of transmission of leprosy to experimental animals.

The diseases leprosy and tuberculosis are closely related in many ways, such as sharing of chemotherapeutic susceptibility and rather broad antigenic relationships in the components of the aetiological agents.

2 Mycobacterium leprae **in Mice: Minimal Infectious Dose, Relationship Between Staining Quality and Infectivity, and Effect of Cortisone,** by CHARLES C. SHEPARD and DOROTHY H. MCRAE. *J. of Bacteriol.* 1965, **89,** 2, 365-372, (14 refs).

The minimal infectious dose of *Mycobacterium leprae* in mouse foot pads was found to be of the order of 10 solidly staining bacilli. In a titration experiment, the actual number found was 3.4 to 34 solid bacilli, and the order of magnitude was confirmed by experience with inocula containing varying numbers of solidly staining leprosy bacilli from mouse passage and from clincial sources. The acid-fast staining quality of leprosy bacilli was related in a useful way to the subsequent rate at which bacillary growth appeared. When the proportion of solidly staining bacilli was high, the calculated generation time was shortest, and the lower the proportion, the longer the generation times. The results were in accord with the hypothesis that all viable bacilli are solid, and that when they die, most of them become non-solid. Varying proportions of the dead bacilli, perhaps up to 10%, remain solid, at least temporarily. The growth curve of *M. leprae* in mice was followed in several experiments with total counts of acid-fast bacteria and determination of the ratio of solid bacilli. What had been called a maximal stationary phase was seen to consist of sequential phases of conversion of solid to non-solid bacilli (death), reappearance of solid bacilli (growth), and conversion of solid to non-solid bacilli (death). When cortisone was administered, leprosy bacilli grew somewhat more slowly during the logarithmic phase, but attained a higher level, especially of solidly staining bacilli.

3 **Lepra Reactions and Basophil Granulation Test,** by DR. B. B. GOKHALE and DR. M. V. JOGLEKAR. *Indian Practitioner*, **7,** 4, April 1964.

The original basophil degranulation test described by Shelley *et al.* 1962, was used by the authors to study reactions caused by the sulphones and spontaneously. It was standardized by studying known cases of allergy to various drugs, e.g. sodium salt of penicillin-G, streptomycinsulphate, and others. Possibly the test will be applied to differentiate between lepra reactions and those precipitated by sulphones.

4 **Contamination of Healthy Mice with Murine Leprosy-like Acid Fast Bacillus,** S. NISHIMURA, Y. KAWAGUCHI, K. KOHSAKA, and T. MORI. *La Lepro*, **33,** 4, 1964, pp. 245-256.

This paper is in English and contains 4 tables and 8 figures in colour. The author found that caution must be exercised in murine experiments of inoculation with the leprosy bacillus because acid-fast bacilli present in natural circumstances must be noted as an infectious agent of natural murine leprosy. Mice were inoculated with organisms isolated from bacillus-positive animals. Leproma was produced and identification tests showed that many of the organisms had properties similar to the murine leprosy bacillus.

This murine leprosy-like acid-fast bacillus has several points of difference, such as the absence of active infection in the original animal, lack of leproma production, proliferation in the lungs in the next generation of mice, and the simple conglomeration of the organism rather than its presence in the cells.

A severe murine leprosy infection was reported in 1932 by Krakower and González in the brown wild house mouse (*Mus musculus*), but other reports are rare. The rarity of natural murine leprosy infection in the mouse compared to the rat may be due to lack of observation, or to an inability of the mouse to survive until the disease can fully develop.

The authors note that the murine leprosy bacillus proliferates only in certain cells of the rat or mouse and cannot grow in any artificial medium and is an obligate cellparasite and is not present widely in nature. Despite this, it is present in the lymph nodes and subcutaneous tissue of apparently healthy mice which have not contacted infected murine leprosy. It is probable that the source of infection is a micro-organism present in the earth. Acid-fast bacilli could enter the body of the rat through a defect in skin or hair follicle; some bacilli may have mutated already so as to grow *in vivo*, and ready to respond to transduction, lysogenic conversion, and other hereditary factors. Further investigations are needed.

When the acid-fast bacillus has succeeded in invading the animal and adapting itself, the question arises why a subcutaneous leproma is not produced. Nishimura found that in an experiment, 9 of the inoculated animals

developed leproma in the next generation in young animals mostly. The survival time of the experimental mouse is short while the generation time of the murine leprosy bacillus is long. This may play some part. Also it can be assumed in nature there is no invasion by a massive quantity of bacteria at one time, but a repeated invasion by small numbers of bacteria so that resistance is gradually built up by the host.

Pronounced pathological changes were found in the lungs in many cases rather than at the subcutaneous site of inoculation, and proliferation was greater. It was found that a leproma constantly was produced in the next generation of mice inoculated with material from the pulmonary lesion, but only a few animals inoculated with subcutaneous material developed leproma. The reason for this difference is not clear. Changes occur early and in a high percentage in the organs, especially the spleen.

The authors consider that the isolated organism is the murine leprosy bacillus, but use the term 'murine leprosy-like acid fast bacillus' in their title of the paper because of the several questions in the process of proliferation of the organism, such as the absence of leproma formation in the first generation of mice inoculated with the material from the original animal, the development of the leproma in the second generation of mice inoculated with material from the pulmonary lesion of the first generation, and finally the simple conglomeration rather than typical cellular proliferation. The authors surmise the presence of some factor by which the acid-fast organism is changed to the murine leprosy bacillus, and propose to call it 'leprosy-like acid-fast-bacillus', until the matter is clarified.

5 **The Need for Bringing Leprosy Research into Universities.** (Address given by DR R. G. COCHRANE, of 57a Wimpole Street, London, Acting President, International Leprosy Association, at a Conference of Research Workers at Washington held under the auspices of The Leonard Wood Memorial, 11 May 1965).

This was a very congenial subject for Dr Cochrane, as he had long emphasized the need for integrating leprosy into the total picture of leprosy research, and he welcomed the opportunity of introducing this subject.

The author does not claim to be a research worker, but insists that he is a clinician deeply interested in research and appreciative of the fact that significant progress in clinical medicine and therapy is absolutely dependent on the fundamental research worker, and has been successful in attracting many such to the problems of leprosy. He has found that scientific research with the rat leprosy bacillus is more readily accepted than with *Myco. leprae*, but the way to a more detailed study is now open since the great work of Shepard, Binford, and Rees. He suggests that there would be profit in extending the investigation of *Myco. leprae* to other animals. Those used so far have been the smaller animals with a life span of about 2 years. Small size of an animal hinders the harvesting of a reasonable number of acid-fast bacilli. Because *Myco. leprae* will grow in the foot pads of mice and hamsters, why should it not grow in the foot pads of larger animals, and animals with longer life span? There is a further potential problem. Growing *Myco. leprae* in a foreign environment may develop in it different characteristics which may be perpetuated. We cannot fail here to stress the importance of the genetic element in leprosy in the human tissue in which *Myco. leprae* grows, and also in the *Myco. leprae* itself. This is a completely new avenue of research. This is best done at University level and in co-operation with the Departments of Human Statistics and Bio-physics and with the assistance of clinicans well versed in leprosy and knowledge of its racial variations (It is well-known that leprosy in Caucasians and Mongoloids is very different clinically from that in Indians and Africans). Such a genetic research project should be long term, and adequately provided for financially. The addition of an epidemiologist would be of great value to the project. For the project, sporadic research would be relatively profitless Dr S. D. Spickett who initiated research on genetic lines said 'The most hopeful approach to leprosy would be the formation of large integrated research groups'.

At the present time university centres do not have a clear understanding of the importance of other scientific disciplines in leprosy. Relatively few persons have adequate training in scientific methods, and on the other hand research workers trained in scientific disciplines often become involved in peripheral matters because their lack of knowledge of leprosy precludes recognition of relevant points. S. D. Spickett also said leprosy research suffers on 2 counts, (1) those doing pure research in leprosy tend to have scanty clinical experience, and (2) those who have clinical experience are out of touch with potential research workers.

In any case the scientific worker has failed to produce regular growth of the micro-organism in *vitro*, so we still cannot study the life history, its metabolic requirements, its growth bye-products, and what is perhaps more important, the products of breakdown at bacillary death.

Over the past century numerous attemps have been made to culture *Myco. leprae*. None has been substantiated. Famous work is that of Doull, McKinley, Sister Marie Suzanne, and Sister Marie de la Trinité. The author thinks that the work of Khanolkar and Ranadive deserves more attention. Khanolkhar used the posterior root ganglia, and later found that the organism grew on a tuberculosis medium. Khanolkar's suggested 'first passage' through the small superficial nerve plexuses of the skin opens up interesting possibilities in the attempt to culture *Myco. leprae*. Dr Ranadive and her colleagues think that it is important to study the behaviour of this bacillus in the foot-pads of mice, and to study lepromin results from it against standard Mitsuda lepromin (histological picture should also be compared, as if the histology tallies there is additional circumstantial evidence that the bacillus is isolated; if they do not tally there is perhaps evidence of a close affinity and the degree of it). The author thinks that in study of *Myco. leprae* one must always remember that a change of environment may produce mutants of the organism.

Margaret Murray of New York University has made an important advance in sub-culturing Schwann cells, and her technique calls for mastery by other workers.

A very great significance attaches to the discovery of lysosomes by De Duve and Novikoff. The presence of these hydrolytic enzymes may explain why relatively so few persons develop leprosy even after most intimate and prolonged contact. There are various other matters that hint at the importance of lysosomes. They may explain,

for instance, why even in children who are constantly exposed to leprosy infection under greatly favourable conditions for the organism, as many as 70% escape infection. Schwann cells are rich in lysosomes. Could it be that natural immunity resides in the Schwann cells? Dr Cochrane suggests that most of the Schwann cells contain enough lysosomal activity to deal with any bacteria which may be introduced into their cytoplasm. Clinically he thinks of non-responding and reacting patients as those possessed of deficient or inhibited lysosomes.

Dr Brieger in his work with electron microscopy found lysosomes within the cells, and when Dr Cochrane heard from him that they had the property of destroying bacteria and bacillary debris, shortly afterward, in a patient with serious relapse Dr Cochrane found with Brieger's aid evidence of absence of lysosomes. This matter also bears on the action of corticosteroids (Dame Honor Fell commented that certain drugs in small doses activate lysosomes and in large doses inhibit lysosomes). Corticosteroids are very powerful in inhibiting lysosomes action. The matter bears on the dosage of sulphones. The author thinks we should reduce it considerably, such as to 10 mgm a week and not exceeding 30 mgm. This succeeds, and Dr S. G. Browne from Uzuakoli agrees. Lessening the dose improved the clinical condition in a recent one of the author's patients, and in a recent relapsed patient there was clear improvement. In a third patient in England, the patient, a chronic one, improved on 30 mgms. DDS a week. These 3 patients were long-standing but the improvement on moderate dosage of sulphones definite.

This opens up a wide field of investigation of lysosomes in Indian and African races. There may be more powerful lysosomal activity, with patients generally less prone to severe reactions and more easy to treat than in Caucasoid and Mongoloid races.

The author says that while *Myco. leprae* is the causative organism of the disease it appears to set up side reactions which make the bacterial invader merely an onlooker who is quite unable to intervene in the disturbances which have been set up. *Myco. leprae* merely serves to trigger a whole series of malignant processes. It is related to autoimmune processes, disorders of pigment etc.

The author comments that he has found in over 90% of patients that the first presenting sign or symptom was anaesthesia. In more and more patients it will be discovered retrospectively in the history. Diagnostic clinics are therefore very important in leprosy campaigns. If diagnosed, early leprosy becomes a controllable incident in life. In some patients in whom leprosy was diagnosed as early as the first presence of bacilli in nerves, it was noted that the disease treatment was highly successful. It would be helpful to find another name for leprosy. A name for the early lesions would be desirable. ('Mycobacterial neuropathic dermatosis' was suggested by J. Ross Innes but ignored largely by those who previously preferred the term Hansen's disease').

6 **Manifestaciones iniciales en la adolescencia y pubertad (Initial manifestations in adolescence and puberty),** DR FÉLIX CONTRERAS, *Revista de Leprología*, 1964, **6,** 2, page 105.

The initial manifestations of leprosy, though they pass unnoticed in most patients, have presented many years before. As long ago as 1797 Pfefferkorn said that leprosy commenced always by a sole and limited lesion in the skin. This same opinion was supported afterwards by Marcano and Wurtz, Leloir, Gougerot, Beurmann, Klingmüller and others.

Brocq in 1907 confirmed the existence of abortive forms which could even regress and not be reproduced, and at that time there was much disagreement between the infective and hereditary theories of the transmission of leprosy. Even it was held that infection could not take place in infancy and Goodhue published the history of some patients who illustrated this.

In fact initial lesions of leprosy were not known until a systematic study took place of the children of leprosy patients born in some leprosaria, especially in the Philippines leprosaria. The first to publish were Rodriguez, Manalang, Velasco and Chiyuto, who maintained that infection almost always took place in children by means of intimate and prolonged contact with the skin of the mother who was a patient. This skin to skin contact resulted in a hypopigmented macule in covered parts. These lesions were apt to coincide anatomically with the maternal bacilliferous lesions and those lesions which had been in intimate and prolonged contact.

Some years later Duarte do Pateo and Solano Lima, Souza Campos, Bechelli and Rotberg, Charria Tovar, Gonzaga, Souza Campos, Bungeler and Alayon, Fernández and other South Americans observed initial lesions, mostly always in children, which were afterwards confirmed by the medical officers of Fontilles. These were observed in patients of Fontilles who lived in some adjacent little towns. Shortly afterwards different doctors of the Spanish provinces began observing such patients, the endemic being more widespread than was thought.

The discovery of early lesions is extremely important, because it allows of easy and certain early treatment of leprosy without scars, and permits of social and public health welfare of patient and family, as well as early control of the infection. The value of early diagnosis is very great, even in less well civilized countries.

The difficulty of infection of adults is recognizable. Even in tuberculosis this is acknowledged, as by Lumiere, who pointed out that most tuberculosis patients were discovered as adults, and least in infancy and youth. The diagnosis must have been late. We should recognize that most of the patients of leprosy start their career in childhood. The greater susceptibility of children is aided by greater susceptibility to heredity (as studied by Aycock and Kinley, and by Fernández.

All leprologists agree in recognizing factors which favour infection and contribute to diminution of resistance to infection. Puberty stands out in these, preferably in women but also to some degree in men. However publications are few of initial lesions in adolescents and in puberty, which doubtless exist, because few are described on entry into military service. We are convinced that mostly these initial lesions pass unnoticed.

We have seen about 200 initial lesions in minors of 16 years, and between 11 and 17 years of age. They were adolescents who were children of leprosy patients, who live all their childhood in leprogenous environment. They showed some defectively pigmented area, and the rest of the skin marked with faint hypo chromia which never

reached the characteristic light macule. The sites were twice in the buttocks, thrice in the thigh muscles, twice on the shoulder, and twice in the forearms and once in the leg. In six patients the plaques coincided with an incomplete alopecia. In all there was sensory change. In six of these and in one other, there was anhydrosis in the hypopigmented lesions. The histamine reaction was incompletely positive. No lepromin reaction nor bacilli were encountered positively.

Recently we have seen the patient A.T.C. of 21 years, unmarried, living in Madrid. He has lived since his birth with his mother and a sister and both were lepromatous patients. He has had good health. Since childhood he thinks he has only had chicken pox. Three months before he attended at the consultation, he noted in the entire surface of the left thigh a small area near the knee that had lost hair and sensitivity, a macule of irregular oval form of 2 or 3 cm., less pigmented than the rest of the skin. There was also some loss of sweating and the histamine test was incomplete. We performed a biopsy which we sent to Félix Contreras Rubio who reported epidermis without histological changes. In the dermis, round some vessels and the nerve filaments there is an inflammatory infiltration consisting of undeveloped lympocitic cells. Bacilli were not encountered; the clinical suspicion of in-determinate leprosy is suggested. We had no doubt that this patient was indeterminate.

We may comment that:

1. For a long time it was thought necessary to find *M. leprae* in our diagnosis of leprosy. I think that the presence of the leprosy bacillus should be guaranteed for a final diagnosis.
2. Yet in indeterminate leprosy it is exceptional to encounter the bacillus.
3. The histology in these cases is limited to a completely non-specific inflammatory reaction, especially round the vessels and the glands.
4. The most characteristic sign of indeterminate leprosy is given by the sensory changes. No other disease exists which produces maculae with anaesthesia.
5. Sometimes also there are changes in sweating and loss of hair. These changes are more evident in adults than in children.
6. In the two adolescents whom I saw, it is probable that the initial changes were analogous to those of childhood, which we know better. Frequently we come to recognise them retrospectively. In some patients useful indicators are disturbance of sweating and tendency of anhydrosis to be limited to a small macule. Pigmentary or sensory changes are useful. I remember one very interesting foreign patient who noted a hypochromic spot in the leg when she was on the beach, when some ants passed over the spot in which she noted a complete absence of sensation just outside the spot which was also lacking in hair.

The most marked symptom according to age consists in lack of pigment which is less marked in adults than in children, and on the other hand lack of sweating and loss of hair are more evident. The most characteristic sign is the anaesthesia which in our opinion is enough to attract the diagnosis, for we repeat that we have not known any other dermatosis with persistent and evident anaesthesia in the macules. There have been hundreds of children who have been saved from the disease by early diagnosis, though we know some of those diagnosed who have not persisted in the treatment and in whom the disease has progressed after some years. Therefore we believe that the discovery of initial lesions is the foundation of prevention in leprosy which should be studied in all contacts especially in children.